INDUCED BREEDING OF *CLARIAS ANGUILLARIS* WITH *XENOPUS LAEVIES* (AFRICAN CLAWED FROG) CRUDE PITUITARY GLANDS.

Tsadu S.M., Yisa A.T.
and
Etuh S.P.

Department of Water Resources, Aquaculture and Fisheries Technology,
School of Agriculture and Agricultural Technology,
Federal University of Technology, P.M.B. 65 Minna
Shabatsadu06@yahoo.com 08035990758

ABSTRACT

Ripe C. anguillaris, size ranging from 350 – 700 g total body weight (TBW), were treated with frog (Xenopus laevis) crude pituitary glands at four treatment levels of 1 pituitary, 2 pituitaries and 3 pituitaries per broodstock of mean weight 434.44 ± 79.39 g and Ovaprim for control. Each treatment was replicated three times. The frogs' weight ranged from 39.40 – 60.00 g. The latency period before successful stripping was 14 hours with one pituitary hormone dosage, 12 hours with two pituitaries and 9 hours with three pituitaries. Eggs were incubated at temperature range of 26 – 27°C. Hatching started after 24 hrs and was completed by 36 hrs of incubation. Egg yield or fecundity was observed to vary according to the dosage of pituitary glands administered. Two pituitary glands injection yielded the highest egg number with mean fecundity of 43749 ± 3005 followed by one pituitary gland treatment with mean fecundity of 34786 ± 5386. Three pituitary glands treatment gave the least fecundity of 26007 ± 4360. Percentage fertilization and hatching of the eggs were also higher in two pituitary glands treatment with 98% fertilization and hatching. This was followed by one pituitary (98% fertilization and 75% hatching) and three pituitary glands with 98% fertilization and 62% hatching. The fries were reared for 8 weeks. Mortality was observed to be highest during the second week in all the three treatments. Treatment two (2 pituitaries) still had the highest survival of 68.40% followed by treatment one (1 pituitary) with 29.60% survival and treatment three (3 pituitaries) with only 4.80% survival. The stock stabilized and no mortality (0%) was recorded from 3rd to 8th weeks of rearing. Results indicated that 2 pituitary glands treatment was most effective followed by 1 and 3 glands treatments respectively. Weight of frogs and their respective pituitaries and weight of fish appeared to have relative effects on their productivity. Three frogs (T_3) of mean weight 67.52 ± 33.70 were observed to be an over dose to a fish of 434.44 g mean weight.

Key Wards: *Clarias anguillaris, breeding, frog pituitary*

INTRODUCTION

The demand for fish fingerlings for aquaculture is on the increase in Africa and has made hatchery propagation of culturable fish species important. Many fish species have been induced to spawn using different inducing hormones, as reported by Manickan and Joy (1989) and Ayson (1991). Some of these inducing agents include carp pituitary gland (Janseen, 1985), Human chorionic gonadotropin (HCG) (Lengendre 1986), Progesterone and LHRHa (Richter *et al.*, 1987) De-oxycorticosterone acetate (DOCA) (Solar *et al.*, 1990). Ovaprim and Ovatide In many developing African Countries these materials are not always available due to marketing problems. This scarcity has led to the search for locally available alternative such as the pituitary gland of the frogs. Nwadukwe (1993) used pituitary extract of frog *Dicroglossus. occipitalis* to induce oocyte maturation, ovulation and spawning in *Heterobranchus longifilis*, Adebayo and Fagbenro (2008) used Bull frog pituitary extract to induce spawning in *Clarias gariepinus*, Adebayo and Popoola (2004) also used frog pituitary gland to induce ovulation in *C. gariepinus*. Mustafa *et al* (1984) spawned the Asian catfish *Hetewropneustes fossilis*

with frog *Rana tigrina* pituitary gland. While the condition of the brood fish and the environmental condition are important the administration of the appropriate hormone is also equally important. The large number of failures in induced breeding can often be traced to inappropriate hormone inducement, poor condition of the brood fish, including their health and nutrition and stage of gonad development as well as to environmental condition in spawning tanks or enclosures (Kutty, 2005). The scarcity of genetically improved fish seed constitutes the major constraint to the rapid development of aquaculture industry and stock management in Nigeria. The mud fish *Clarias anguillaris* species are species of economic importance in Nigeria. They are widely cultured owning to their hardiness, early maturity and good market price (Nwadukwe 1993).

African clawed frog (*Xenopus laevis*) is a giant species that grows up to between 100 – 130mm in length, with adult males generally 10 – 30% smaller (Gamper 1995). They are air breathing aquatic frog that occurs in virtually every water body in its native range of Sub-Saharan Africa. This frog is most commonly found in stagnant or still waters of ponds or sluggish streams but may also inhabit fast flowing waters (Gampper, 1995).

The sacrifice of male Clarias species for milt often leads to depletion of male brood stock from fish farms or hatcheries. The use of frog pituitary to induce breeding in this species has not been a common practice in Nigeria. Evolutionary evidence from morphological and physiological characteristics has indicated that frogs (Amphibian) and fish (Pisces) have the same ancestral origin (Roberts 1975). There is no known standard dosage of frog pituitary for fish breeding. This work is also a pioneer work on breeding of *Clarias anguillaris* using frog crude pituitary. The objectives are: to investigate the effectiveness of frog (*Xenopus laevis*) pituitary gland in induced breeding of *Clarias anguillaris* with the view to cut down the cost of fingerling production from rather expensive second generation hormone compounds; to determine the hatchability of *Clarias anguillaris* eggs induced with frog pituitary gland and to establish a dosage of frog crude pituitary gland to be recommended for breeding of *Clarias anguillaris*.

MATERIALS AND METHODS

The experiment was conducted at the hatchery complex of school of Agriculture and Agricultural Technology Fish farm unit, Federal University of Technology. Minna. Twelve female and four male *Clarias anguillaris* broostocks, size ranging from 350 – 700 g in weight were used for the experiment. Eighteen (18) live frogs (*Xenopus laevis*), size ranging from 39.40 – 60.00 g collected from River Gadu in Minna were used for the experiment. Four treatments including control were carried out in the following experimental design. One frog pituitary gland to one female fish, replicated three times as treatment 1; two frog pituitaries to one female fish, replicated three times as treatment 2; and three frog pituitaries to one female fish, replicated three times as treatment 3. The control group were treated with Ovaprim at 0.5 mgL^{-1} /kg. The frogs' weights and weights of pituitary glands extracted were recorded. The pituitary glands were macerated and homogenised in 0.5 ml saline solution in a laboratory crucible and administered intraperitonially to the fish using 1 ml syringe and needle. After injection the fish were placed in hatchery tanks, 1x 1.5 m in size and observed for latency period during which ovulation was tested for by test stripping at every two hours. After the latency period, eggs were stripped and fertilized normally by wet technique with milt from the male donors. Four males were sacrificed for their milt, one for each set of treatment. Fecundity of each female treated was determined by the method of Bagenal (1978). After fertilization the eggs were incubated for 24 hrs. Percentage fertilization and hatching were estimated from the number of unhatched and hatched eggs. The four treatments were carried out separately as three breeding trials with two days intervals for incubation and hatching of each set. After hatching the fry were reared in glass aquaria tanks for 8 weeks during which mortality, survival and growth rates (increase in length and weight) were recorded. The data was used to determine percentage mortality, survival and growth rates. Results were compared by one-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Induced breeding of *C. anguillaris* with frog pituitary gland was successfully carried out at four

treatment levels including control. The latency period before successful striping ranged from 9 – 14 hours at ambient temperature of 26 -27°C. This fall within the range observed by Nwadukwe (1993) which was 11 hours at 25°C, he also reported that it reduced to 7 hours at 29°C. Sule (1999) observed that the optimum latency period before stripping Clarias species in arid zones of Nigeria is 9 hours. Hatching started after 24 hours and was completed by 36 hours at 26 – 27°C. Commencements of hatching at 24 hours of incubation have been observed by others including Manikan and Joy (1989) and Nwadukwe (1993). Fecundity varied according to the hormone dosage and treatment. Two pituitaries combined (T2) gave the highest egg release and mean fecundity of 43749 ± 3005 followed by one pituitary treatment (T1) with mean fecundity of 34786 ± 5386. Three pituitaries combined (T3) gave the least mean fecundity of 26008 ± 4360. Two pituitaries combined weighing 0.5g was the most effective dosage followed by one pituitary weighing 0.42g. Three frog pituitaries (T3) with mean weight of 0.62g appeared to be an overdose to fish of 550g mean weight. Nwadukwe (1993) achieved oocyte maturation ovulation and hatching with frog pituitary dose of 7 mg/ kg fish weight. Percentage fertilization was 98% in T1, T2 and T3 and 96% in the control. Percentage hatching was highest in T2 98% followed by T1 75%, control 75% and T3 62%. Nwadukwe (1993) obtained mean % fertilization of 73.50 ± 9.30% and mean hatching of 63.08 ± 7.08%. Table 1 shows the analysis of variance (ANOVA) for comparison of fecundity, % fertilization and hatching, initial and final weight of fry reared for eight weeks. Results of water quality parameters analysis are presented in Table 2. The values are within the tolerance range for hatching, survival and growth of fish as reported by Marylin (1976), Viveen (1986) and Ayinla (1991).

CONCLUSION AND RECOMMENDATION

The experiment has indicated that hypophysation with frog pituitary gland can successfully induce breeding in *C.anguillaris* at a dosage of 2 frog pituitaries (0.5mg pituitary/0.5Kg fish). The dosage was

Table 1: Analysis of variance (ANOVA) for comparison of fecundity, % fertilization, % hatching and initial and final weight of fry produced from *C.anguillaris* induced with frog pituitary and reared for 8 weeks.

Parameter	Treatment 1	Treatment 2	Treatment 3	Control	+S.E.
Fecundity	34786 ± 5386^{c}	43749 ± 3005^{d}	26008 ± 4360^{a}	30444 ± 1054^{b}	6089.94
% Fertilization	98.90 ± 0.00^{a}	98.80 + 0.00^{a}	98.20 ± 0.00^{a}	96.20 ± 2.46^{b}	1.422
% Hatching	75.50 ± 0.00^{b}	98.60 + 0.00^{c}	62.30 ± 0.00^{a}	75.67 ± 2.55^{b}	1.453
Mean initial weight of Fry(g)	3.66 ± 2.88^{a}	4.16 ± 2.89^{ab}	5.20 ± 2.11^{c}	4.50 ± 3.22^{b}	7.63
Mean Final weight of Fry(g)	33.90 ± 8.69^{a}	38.66 ± 9.62^{b}	32.33 ± 7.24^{a}	35.66 ± 32.11^{ab}	7.62
Mean weight gain (g)	30.24 ± 5.81^{b}	34.50 ± 6.73^{c}	27.13 ± 5.13^{a}	31.16 ± 28.89^{b}	1.45

NB: Letters in the same row carrying the same superscript indicate no significant difference

Table 2: Weekly mean water quality parameters of water used to rear fry produced from *anguillaris* induced with frog pituitary gland extract.

Weeks	Temperature (°C)	pH	Dissolved Oxygen(mgL^{-1})	Conductivity (µohm/s)
1	28.1 ± 2.40	6.81 ± 0.52	6.2 ± 0.45	111.7 ± 12.4
2	28.0 ± 2.40	7.02 ± 0.68	6.0 ± 0.41	79.5 ± 6.02
3	26.0 ± 2.25	6.98 ± 0.55	5.6 ± 0.38	90.1 ± 8.50
4	26.0 ± 2.25	6.82 ± 0.51	6.4 ± 0.48	85.2 ± 7.03
5	27.0 ± 2.35	6.42 ± 0.25	6.2 ± 0.45	108 ± 11.02
6	26.0 ± 2.25	6.94 ± 0.53	5.6 ± 0.38	165.1 ± 15.20
7	25.0 ± 2.05	6.97 ± 0.55	6.0 ± 0.41	174 ± 15.51
8	26.0 ± 2.25	6.85 ± 0.52	5.8 ± 0.39	201 ± 18.05

REFERENCES

Adebayo O.T. and Fagbenro O.A. (2008) Effect of storage period on the efficacy of African bull frog pituitary extract for induced spawning of *C. gariepinus. International Journal of Zoological Research* 4: 77–80.

Adebayo, O.T. and Popoola, O.M. (2004) Storage period; its effect on efficacy of non-piscine (Frog) hormone used in inducing ovulation in African catfish *C. gariepinus International Journal of Zoological Research* 4(2): 124–128.

Ayinla O.A. (1991): Feed Requirement and Fattening of Broodstock *Proceedings of the Fish Seed propagation Course* Africa Regional Aquaculture Centre (ARAC) Aluu Port Harcourt Nig. p.20.

Ayson F.G. (1991) Induced Spawning Rabbit Fish, *Siganusguttatus* (Bloch) Using Human Chorionic gonadotropins (HCG). *Aquaculture* 95, 133-137.

Bagenal, T.B. (1975) *Methods for assessment of fish production,* Blackwell Scientific Pub LtD. Oxford OX2 OEL 155–160

Gampper, T. (1995) (September 21, 2001) *Natural History of the upland Clawed Frog,* Nebraska Herpetological Society: http//wwwsonic.net/-melissk/xenopushtml. P 1-10

Janseen J.A.L (1985) Elevage du poisson-chat Africain, *Clarias lazera* (Cuv & Val 1840) en Republique Centafricain. 1: Propagation Artfiicielle. *FAO Project GCP/CAR/007/NET Banui*; C.A.R. DOC. Tech. 20, 100pp

Kutty, M.N. (2005): *Aquaculture Principles and Practices* Second Edition. FAO/NACA, Aquacultural Expert and Black Well Publishing Limited, 9600 Gersington Road, Oxford Ox4 2DQ UK pp. 174-179.

Legendre M. (1986) Seasonal Changes in sexual Maturity and Fecundity and HCG Induced Breeding of the catfish, *Heterobranchus Lonifilis Val.* (Clariidae) Reared in Ebric Lagoon (Ivory Coast). *Aquaculture* 55, 201-213.

Manickan P. & Joy K.P. (1989), Induction of Maturation and Ovulation by Pimozide-LHRH Analogue Treatment and Resulting Higher Quality Egg Production in Asian Catfish *Clarias barachus. Aquaculture* 83, 193-199.

Marilyn, C. (1976). *Freshwater Fish Pond Culture and Management Appropriate Technology for Development* Pp, 47–50.

Mustafa, S., Ahmed, Z., Murad, A. and Zofair, S.M. (1984) Induced spawning of catfish by frog pituitary gonadotropin. *Progresive Fish Culturist* 46: 43–44.

Nwadukwe, F.O. (1993). Induced oocyte maturation, ovulation and spawning in the African catfish, *Heterobranchus longifilis* using frog pituitary extract *Journal of Aquaculture and Fisheries Management* 24: 625–630.

Richter AC.J.J. Eding E.H, Goss H.L.Th. De LeeuwR, Scott A.P and Van Oordt P.G.W.J. (1987). The Effect of Pimozide/LHRHa and 17a hydroxyprogesterone on Plasma Steroid Levels and Ovulation in the African Catfish *Clarias gariepinus Aquaculture* 63, 157-168

Roberts, M.V.B (1975) *Biology: A Functional Approach.* Reconstructing evolutionary pathway Pp 552 553.

Solar L.L. Mclean E. Baker I.j. Sherwood N.M. & Donaldson E.M. (1990). Induced Ovulation of Sable Fish (*Anoploma Fimbria*) (D-Ala^6) LH-RH Ethylamide *Fish Physiology and Biochemistry* 8, 497-499.

Sule, O.D. (1999) Determination of Optimum Latency time for Stripping *Clarias gariepinus* in the Arid zone of Nigeria, *National Institute for Freshwater Fish Research (NIFFR) Annual Report.* P. 73

Viveen, W.J., A.R., Richter, C.J.J., Van Oordt, (1986). *Practical Manual for the Culture of the African Catfish* Netherlands Pub. *Clarias gariepinus* Pp 1-10